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Assessment of Regression Models for Adjustment of Iron Status Biomarkers for Inflammation in Children with Moderate Acute Malnutrition in Burkina Faso[1–3]

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Abstract

Background: Biomarkers of iron status are affected by inflammation. In order to interpret them in individuals with inflammation, the use of correction factors (CFs) has been proposed.

Objective: The objective of this study was to investigate the use of regression models as an alternative to the CF approach.

Methods: Morbidity data were collected during clinical examinations with morbidity recalls in a cross-sectional study in children aged 6–23 mo with moderate acute malnutrition. C-reactive protein (CRP), α_1-acid glycoprotein (AGP), serum ferritin (SF), and soluble transferrin receptor (sTfR) were measured in serum. Generalized additive, quadratic, and linear models were used to model the relation between SF and sTfR as outcomes and CRP and AGP as categorical variables (model 1; equivalent to the CF approach), CRP and AGP as continuous variables (model 2), or CRP and AGP as continuous variables and morbidity covariates (model 3) as predictors. The predictive performance of the models was compared with the use of 10-fold crossvalidation and quantified with the use of root mean square errors (RMSEs). SF and sTfR were adjusted with the use of regression coefficients from linear models.

Results: Crossvalidation revealed no advantage to using generalized additive or quadratic models over linear models in terms of the RMSE. Linear model 3 performed better than models 2 and 1. Furthermore, we found no difference in CFs for adjusting SF and those from a previous meta-analysis. Adjustment of SF and sTfR with the use of the best-performing model led to a 17% point increase and <1% point decrease, respectively, in estimated prevalence of iron deficiency.

Conclusion: Regression analysis is an alternative to adjust SF and may be preferable in research settings, because it can take morbidity and severity of inflammation into account. In clinical settings, the CF approach may be more practical. There is no benefit from adjusting sTfR. This trial was registered at www.controlled-trials.com as ISRCTN42569496. *J Nutr* 2017;147:125–32.

Keywords: inflammation, α_1-acid glycoprotein, correction factors, C-reactive protein, iron deficiency, regression analysis, serum ferritin, soluble transferrin receptor, young children

Introduction

Anemia is a major public health issue and affects an estimated 71% of young children <5 y of age in western and central Africa (1). It can cause fatigue and has been associated with poor cognitive and motor development (2). Iron deficiency (ID)[8] is believed to be responsible for 50% of anemia cases (3). Other

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[3] Supplemental Table 1 is available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

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[8] Abbreviations used: AGP, α_1-acid glycoprotein; APP, acute-phase protein; CF, correction factor; CRP, C-reactive protein; GAM, generalized additive model; ID, iron deficiency; MUAC, midupper arm circumference; RMSE, root mean square error; SF, serum ferritin; sTfR, soluble transferrin receptor.

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causes of anemia include infectious diseases, hemoglobinopathies, and deficiencies of folate, vitamin B-12, or vitamin A (2, 4).

Diagnosis of ID is necessary for a better understanding of the causes of anemia, identifying individuals who are most likely to benefit from iron supplements, and evaluating the effectiveness of interventions to combat anemia. It is, however, a challenge because biomarkers of iron status, namely serum ferritin (SF) and serum soluble transferrin receptor (sTfR), are affected by inflammation (4, 5). More specifically, SF acts as a positive acute-phase reactant (6). sTfR is believed to be less affected by inflammation (4), although there are discrepancies in the literature regarding the relation between inflammation, infection, and sTfR. Some studies have shown that sTfR decreases in the presence of inflammation (6, 7) and malaria (8), whereas others found higher concentrations of sTfR in individuals with malaria (9, 10) or observed positive relations between inflammation markers and sTfR (10–14). It is unclear what causes these discrepancies, but they may be due in part to different levels of immunity, time course and severity of infection, and the infection causing the inflammation and anemia.

In order to interpret biomarkers of iron status in the presence of inflammation, Thurnham et al. (15, 16) have suggested applying correction factors (CFs) to measured concentrations of SF in individuals with inflammation, defined as elevated serum concentrations of the acute-phase proteins (APPs) serum C-reactive protein (CRP) and/or serum α_1-acid glycoprotein (AGP). Although it is widely accepted that SF needs to be adjusted for inflammation (10, 14, 15, 17–21), it is still debated whether it is necessary to adjust sTFR (10, 21–23).

The CF approach is easy to apply and has been used in a number of studies (10, 14, 17–20). However, it relies on single cutoffs, and therefore ignores the fact that the impact of inflammation on biomarkers of iron status depends on the severity of the inflammation (11, 23) and may also depend on the cause of inflammation. In contrast, regression modeling, which has been proposed as an alternative to the CF approach (24), is not dependent on cutoffs, and has the advantage that it can take morbidity covariates into account. It therefore may be a better option in populations with a high prevalence of infections. One concern about the use of linear regression models is that relations between APPs, namely CRP and AGP, and biomarkers of iron status are not linear (24); thus, it may be necessary to use more flexible regression models. Regression models previously have been used to adjust for inflammation (22, 23), but more studies are needed, in particular in contexts in which infections as well as malnutrition are common.

The objective of this study (ISRCTN42569496) was to investigate the use of regression models in adjusting biomarkers of iron status for the effect of inflammation in young children with moderate acute malnutrition in Burkina Faso, where, as previously shown, inflammation and morbidity are common (25).

Methods

Study area and population. The data for this paper were baseline data collected as part of the Treatfood trial, a randomized trial with the objective of assessing the effectiveness of 12 supplementary foods for treatment of moderate acute malnutrition, defined as a weight-for-height *z* score between –3 and –2 and/or a midupper arm circumference (MUAC) between 115 and 125 mm. As previously described (26), the trial was carried out in 5 health centers in the Province du Passoré, Burkina Faso. The study catchment area covered a total of 143 villages and a total population of ~258,000.

Children aged 6–23 mo with moderate acute malnutrition who were residents in the catchment area and whose parents or guardians provided consent for their children to participate were included. Children who were hospitalized or treated for severe acute malnutrition in the previous 2 mo, children with hemoglobin <5 g/dL, children who were already enrolled in a nutritional program, and those who had medical complications requiring hospitalization were not included. Screening for participants was carried out by community health workers with the use of MUAC tapes and designated screening teams that used both MUAC and weight-for-height *z* score. In addition, children could be referred from a health center or could present on site at the initiative of the caregiver. Recruitment took place from September 2013 until August 2014.

Data collection. Sociodemographic data were collected by trained interviewers. Body weight was measured to the nearest 0.1 kg with the use of an electronic scale with double weighing function (Seca model 881 1021659). Length was measured to the nearest 0.1 cm with the use of a standard UNICEF wooden measuring board. All children were measured while lying down. MUAC was measured on the left arm to the nearest 1 mm. During clinical examinations and 14-d retrospective morbidity interviews, research nurses collected the following morbidity data: rash, skin infection, runny nose, cough, ear discharge, upper respiratory infection, lower respiratory infection, diarrhea, fever, and malaria, as well as history of fever, cough, diarrhea, vomiting, rash and swelling. Venous blood (2.5 mL) was collected from the arm. One drop was used for diagnosis of malaria with the use of a rapid diagnostic test that detects histidine-rich protein 2 synthesized by the *Plasmodium falciparum* malaria parasite (Bioline, Malaria Ag P.f; Standard Diagnostics), and 1 drop of blood was used to estimate hemoglobin concentration with the use of a HemoCue device (HB 301). The HemoCue was calibrated at the end of every month with a control solution. The remaining blood was added to a sample tube with clot activator (BD reference no. 368492) and transported to the trial lab in a cold box at 2–8°C. Serum was isolated after centrifugation at 700 × *g* for 5 min (EBA 20 S; Hettich) at room temperature and stored at −20°C until shipment to the VitMin Lab in Willstaedt, Germany, for analysis of CRP, AGP, SF, and sTfR with the use of a combined sandwich ELISA (27). All samples were measured in duplicate, and both intra- and interassay CVs were <10%. Samples were frozen and thawed only once before analysis.

The thresholds used for defining abnormal values were as follows: hemoglobin <11 g/L (28); SF <12 μg/L (28); sTfR >8.3 mg/L (27); CRP >5 mg/L (24); AGP >1 g/L (24). Fever was defined as an axillary temperature ≥37.5°C. Upper and lower respiratory tract infections were diagnosed by experienced pediatric nurses on the basis of an adapted version of the Integrated Management of Childhood Illnesses guidelines (29, 30). Diarrhea was defined as ≥3 loose watery stools/d.

Data handling and statistical analysis. Data were double entered into Epidata 3.1 software, and double-entry checks were carried out on a daily basis. All statistical analyses were carried out with the use of the statistical software R (31). P values < 0.05 were considered to be significant.

Characteristics of the study population were summarized as percentages, means ± SDs, or, if not normally distributed, as medians (IQRs). Scatter plots with a best-fitting local regression curve were used to display any possible nonlinear relations between biomarkers of iron status and APPs.

Three types of models were used to predict logarithm-transformed SF and sTfR, namely, generalized additive models (GAMs), which flexibly allow modeling of nonlinear relations, quadratic models, and linear models. For each of these 3 model types, 5 models for each iron status biomarker as outcome were built with *1*) CRP as a continuous variable, *2*) AGP as a continuous variable, *3*) CRP and AGP as continuous variables, *4*) both APPs and morbidity covariates, or *5*) inflammation groups as independent variables. The inflammation groups used were no inflammation, incubation (CRP >5 mg/L only), early convalescence (CRP >5 mg/L and AGP >1 g/L), and late convalescence (AGP >1 g/L only), as previously described by Thurnham et al. (15). Stepwise backward elimination was used for variable selection. The first 4 models were fitted to the subset of the data that consisted of individuals who had a CRP >5 mg/L and/or AGP >1 g/L, and the last model was built in the full dataset, because the base category was children without inflammation. Model checking was based on residual and normal probability plots.

The predictive performance of the models was compared with the use of 10-fold crossvalidation. More specifically, the data set was randomly split into 10 subsets of equal size. In turn, each of these subsets of the data set (test set) was left out, and models were fitted to the remainder part of the data (training set). For both SF and sTfR, predictive performance was evaluated with the use of root mean squared errors (RMSEs) between observed and predicted values, where a lower RMSE indicated better performance.

After the crossvalidation, adjusted SF and sTfR concentrations were calculated with the use of regression coefficients from the models. As an example, the formula for calculation of adjusted SF concentrations with the use of the model with both CRP and AGP as independent variables would be as follows: Adjusted SF = exp(log SF − β_{CRP} × CRP − β_{AGP} × AGP), where β_{CRP} is the regression coefficient from the model and logSF is logarithm transformed SF.

Only concentrations in individuals with CRP >5 mg/L and/or AGP >1 g/L were adjusted. Because back-transformed regression coefficients from logarithm-transformed models are equal to the ratio of geometric means, the model with inflammation groups as an independent variable corresponds to the CF approach previously described by Thurnham et al. (15, 16), where ratios of geometric means are converted to correction multipliers by dividing 1 by the ratio. We compared our results with the ratios calculated in a meta-analysis (15) for both infants (<12 mo of age) and children (<18 y of age) with the use of approximate *t* tests. Prevalence of ID was calculated for unadjusted and adjusted values, as well as separately for individuals with and without inflammation based on the cutoffs for SF and sTfR mentioned above.

TABLE 1 Characteristics of 1564 children aged 6–23 mo with moderate acute malnutrition in Burkina Faso[1]

Characteristic	Value
Sex, M	45.1 (706)
Age, mo	11.4 [8.2–16.2]
Anthropometric measurements	
Inclusion category	
Low MUAC[2] only	29.0 (454)
Low WHZ[3] and low MUAC[2]	50.1 (784)
Low WHZ[3] only	20.8 (326)
HAZ <−2	37.7 (590)
Morbidity	
Illness according to maternal recall[4]	37.5 (587)
Illness according to physical examination	71.6 (1121)
Malaria[4]	40.2 (626)
Laboratory tests	
Serum CRP, mg/L	2.3 [0.8–9.4]
0–5	64.1 (1002)
>5–10	11.7 (183)
>10–20	9.2 (144)
>20–40	7.0 (110)
>40	8.0 (125)
Serum AGP, g/L	1.2 [1.19–1.24]
0–1	33.0 (517)
>1–2	52.4 (819)
>2–3	10.8 (169)
>3	3.8 (59)
Hemoglobin, g/L	10.0 ± 1.6
<11	70 (1095)

[1] Values are % (*n*) for categorical variables, means ± SDs for continuous variables with a normal distribution, or medians [IQRs] for continuous variables with a skewed distribution. AGP, α_1-acid glycoprotein; CRP, C-reactive protein; HAZ, height-for-age z score; MUAC, midupper arm circumference; WHZ, weight-for-height *z* score.
[2] MUAC ≥115 mm and <125 mm.
[3] WHZ ≥−3 and <−2.
[4] Data missing: Ill according to maternal recall, *n* = 9; malaria, *n* =6.

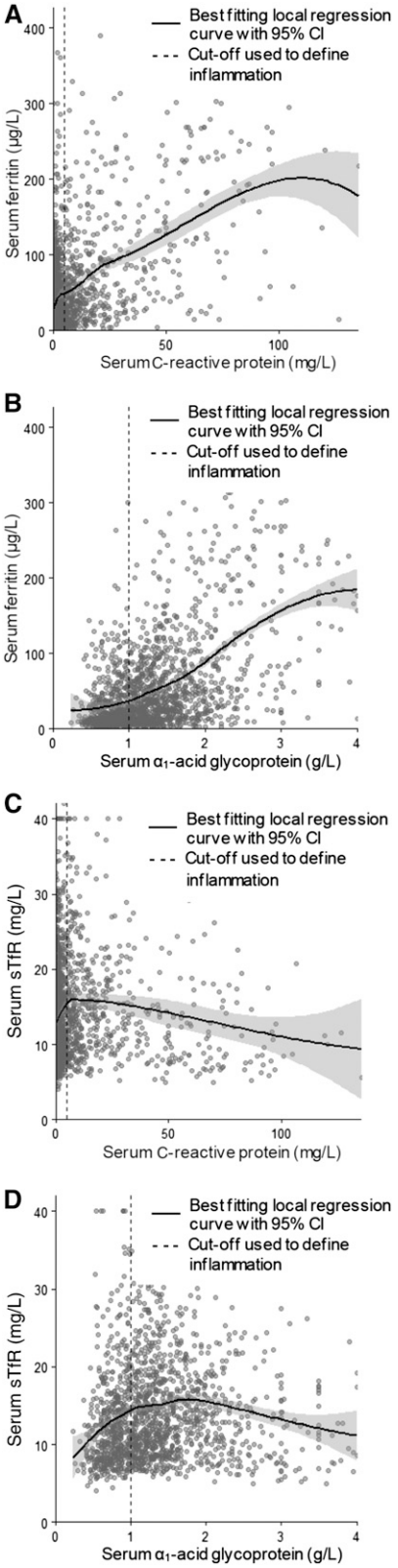

FIGURE 1 Relation between acute-phase proteins and biomarkers of iron status in 1564 children aged 6–23 mo. Relation between CRP and SF (A); relation between CRP and sTfR (B); relation between AGP and SF (C); and relation between AGP and sTfR (D). The gray dots represent serum concentrations of iron status biomarkers (SF or sTfR). The solid black line is the best-fitting local regression curve with 95% CI. The dotted line indicates the cutoff used to define inflammation, i.e., 5 mg/L for CRP and 1 g/L for AGP. AGP, α_1-acid glycoprotein; CRP, C-reactive protein; SF, serum ferritin; sTfR, soluble transferrin receptor.

Ethical considerations. The study was approved by the Ethics Committee for Health Research of the government of Burkina Faso (2012–8-059), and consultative approval was obtained from the Danish National Committee on Biomedical Research Ethics (1208204). The study was carried out in accordance with the Declaration of Helsinki. All children recruited in need of medical treatment received treatment free of charge according to an adapted version of the Integrated Management of Childhood Illnesses guidelines (29, 30) and national protocol. Consent was obtained from caregivers before inclusion, verbally and in writing (signature or fingerprints). Data were kept confidential and in a locked facility.

Results

Sample population characteristics. As previously reported, 1609 children were enrolled in the Treatfood study (25). Among these, 1564 children (82.1%) had baseline SF and sTfR data and were included in the analysis presented here. Background characteristics are presented in **Table 1** and have been described in more detail elsewhere (25). As previously reported, infections and inflammation were common (25). More than two-thirds of children had a symptom or infection diagnosed during the physical examination, 35.8% (n = 561) had elevated CRP concentrations, and 66.4% (n = 1039) had elevated AGP concentrations (Table 1). Only 11.1% (n = 174) of children did not have any inflammation, history of illness, or infections. Anemia was also common (Table 1).

Model selection: SF. Although the relation between SF and APPs was not completely linear, as shown in **Figure 1** A and B and also confirmed by the GAM (P value of smooth terms < 0.05), SF appeared to steadily increase for APP values above the cutoff indicating inflammation, and leveled off at high concentrations of the APPs (Figure 1 A and B). In line with the latter observation, crossvalidation revealed no advantage of using more complex GAMs and quadratic models over linear models in terms of the RMSEs, which were 0.953, 0.952, and 0.957 for the GAMs and quadratic and linear models, respectively, with APPs in continuous form as predictors. Because there appears to be no gain from using more complex models, the remainder of the analysis was based on linear models. Although model type did not greatly affect the predictive performance of the models, the choice of covariates had more of an impact. The RMSEs were reduced if both APPs were included as continuous rather than categorical variables, and they were further reduced if morbidity data were included in addition to APPs (**Table 2**). APPs, malaria, and lower respiratory tract infection, as well as history of fever, were significantly associated with increased log SF concentrations (Table 2). RMSEs for models not presented in Table 2 can be found in **Supplemental Table 1**.

Model selection: serum sTfR. Similarly to SF, the relation between sTfR and APPs was not completely linear, as demonstrated by Figure 1 C and D and confirmed by the GAM (P value of smooth terms < 0.05). sTfR concentrations appeared to steadily decrease as CRP increased for CRP concentrations >5 mg/L. There appeared to be an inverted U-shaped relation between sTfR and AGP, with the apex at an AGP concentration of ~1.5 g/L (Figure 1 D). In line with the observation that sTfR concentration appeared to decrease in individuals with inflammation, crossvalidation revealed no advantage of using more complex GAMs and quadratic models over linear models in terms of the RMSEs, which were 0.426, 0.427, and 0.429 for the GAMs and quadratic and linear models, respectively, that included APPs in continuous form as predictors. The remainder of the analysis was therefore based on the linear models. RMSEs for models not presented in Table 2 can be found in Supplemental Table 1. Similarly to the SF models, the sTfR models performed better if APPs were included in continuous as opposed to categorical form, and performance was further improved if morbidity covariates were added (Table 2). When both CRP and AGP were included in the models, only CRP remained significant. CRP was associated with a decrease in sTfR, whereas malaria, fever, and acute diarrhea were associated with higher concentrations of sTfR (Table 2).

TABLE 2 Prediction models for log-transformed SF and sTfR in 1564 young children from Burkina Faso[1]

	Log SF[2,3]			Log serum sTfR[3,4]		
	Coefficient (95% CI)	P	RMSE[5]	Coefficient (95% CI)	P	RMSE[5]
Model 1: inflammation categories			1.027			0.432
CRP >5 mg/L	0.253 (−0.11, 0.625)	0.2		0.142 (−0.014, 0.299)	0.07	
CRP >5 mg/L and AGP >1 g/L	1.094 (0.969, 1.220)	<0.001		0.149 (0.096, 0.202)	<0.001	
AGP >1 g/L	0.432 (0.305, 0.559)	<0.001		0.147 (0.094, 0.201)	<0.001	
Model 2: acute-phase proteins in continuous form			0.957			0.429
CRP	0.015 (0.012, 0.018)	<0.001		−0.003 (−0.004, −0.002)	<0.001	
AGP	0.454 (0.338, 0.571)	<0.001		—		
Model 3: acute-phase proteins in continuous form and morbidity			0.927			0.410
CRP	0.014 (0.010, 0.017)	<0.001		−0.004 (−0.006, −0.003)	<0.001	
AGP	0.348 (0.232, 0.463)	<0.001		—		
Malaria	0.426 (0.310, 0.541)	<0.001		0.259 (0.209, 0.309)	<0.001	
Lower respiratory tract infection	0.139 (0.008, 0.269)	0.04		—		
History of fever	0.316 (0.177, 0.455)	<0.001		—		
Fever	—			0.072 (0.009, 0.136)	0.03	
Acute diarrhea	—			0.132 (0.029, 0.234)	0.01	

[1] AGP, α_1-acid glycoprotein; CRP, C-reactive protein; RMSE, root mean square error; SF, serum ferritin; sTfR, soluble transferrin receptor.
[2] Model 1: adjusted R^2 = 0.159; model 2: adjusted R^2 = 0.238; model 3: adjusted R^2 = 0.293.
[3] SF was measured in μg/L, and sTfR in mg/L.
[4] Model 1: adjusted R^2 = 0.023; model 2: adjusted R^2 = 0.023; model 3: adjusted R^2 = 0.113.
[5] From 10-fold crossvalidation.

TABLE 3 Comparison of study-generated and meta-analysis geometric mean ferritin ratios for inflammation groups and group with no inflammation[1]

	Study generated	Meta-analysis[2]			
	(n = 1564)	Infants (n = 1278)	P[3]	Children (n = 3695)	P[3]
CRP >5 mg/L vs. no inflammation	1.29 (0.89, 1.87)	1.13 (0.9, 1.41)	0.54	1.56 (1.22, 1.99)	0.36
CRP >5 mg/L and AGP >1 mg/L vs. no inflammation	2.99 (2.63, 3.39)	2.09 (1.66, 2.63)	0.006	2.55 (1.37, 4.72)	0.61
AGP >1 mg/L vs. no inflammation	1.54 (1.36, 1.75)	1.42 (1.14, 1.76)	0.52	1.53 (1.15, 2.04)	0.97

[1] Values are ratios (95% CIs). AGP, α_1-acid glycoprotein; CRP, C-reactive protein.
[2] Geometric mean ferritin ratios for infants aged <12 mo and children aged <18 y from a meta-analysis carried out by Thurnham et al. (15).
[3] Based on approximate t tests.

Comparison of study-generated and meta-analysis CFs for adjusting SF. The ratio of geometric means between the reference and the inflammation groups did not differ from the ones calculated in children in the meta-analysis by Thurnham et al. (15). The ratio for the group with elevated CRP and AGP compared with the group without inflammation generated based on our data was different from the one calculated by Thurnham et al. (15) for the subgroup of infants (<12 mo of age), but did not differ for the other 2 groups (**Table 3**). However, if a comparison was made only based on infants <12 mo of age in our data as well, this difference disappeared (data not shown).

Impact of adjustment on estimated prevalence of ID. Adjusting SF concentrations for the impact of inflammation and infection led to a lower mean SF and a higher estimated prevalence of ID in the sample by 12, 14, and 17 percentage points for model 1 (linear model with inflammation categories as predictor), model 2 (linear model with APPs as continuous variables), and model 3 (linear model with APPs as continuous variables and morbidity covariates), respectively (**Table 4**). The impact of the adjustment of SF is also shown in **Figure 2** A and B. The estimated prevalence based on adjustment with the use of models 2 or 3 was very close to the prevalence of ID in the subset of children without inflammation and without inflammation and/or infection (Table 4). The estimated prevalence calculated with the use of model 1, which corresponds to the CF approach, was slightly lower than that based on the other 2 models. Adjusting sTfR concentrations reduced the prevalence of ID by 7 percentage points based on model 1 and increased the prevalence of ID by 3 and <1 percentage points when based on model 2 and model 3, respectively (Table 4). As also demonstrated in Figure 2 C and D, the impact of adjustment on sTfR was therefore small.

Discussion

Our results confirm that the relations between the 2 APPs, CRP and AGP, and biomarkers of iron status are not completely linear. Nevertheless, linear models perform well and there was no advantage in using the more complex quadratic model or GAMs to predict SF and sTfR concentrations.

To adjust SF for inflammation, the use of regression models is an alternative, and it may be preferable to the CF approach for several reasons. First, the relation between the APPs and SF is fairly linear for concentrations above thresholds used to indicate inflammation, and in terms of predictive performance, there does not appear to be any advantage of using more flexible models. Second, we observed higher SF with increasing severity of inflammation; as a result, models performed better if CRP and AGP were treated as continuous rather than dichotomous variables. Third, although the difference in RMSE between model 2 and 3 and resulting prevalence of ID was small, the results indicate that including morbidity leads to a more precise

TABLE 4 Estimated prevalence of ID with and without adjustment in 1564 6–23 mo old children with moderate acute malnutrition[1]

		SF, μg/L		Serum sTfR, mg/L	
	n	Median (IQR)	ID,[2] % (n)	Median (IQR)	ID,[2] % (n)
Without adjustment					
All participants	1564	33.4 (13.5–74.0)	21.0 (329)	12.6 (9.1–17.3)	82.9 (1296)
Participants with inflammation (CRP >5 and/or AGP >1)	1070	44.4 (18.9–91.6)	14.7 (157)	13.3 (9.7–18.2)	85.7 (917)
Participants without inflammation	494	18.9 (9.5–40.4)	34.8 (172)	11.2 (8.4–15.3)	76.7 (379)
Participants without inflammation and/or illness	174	15.4 (9.3–29.2)	38.6 (66)	8.14 (8.05–8.23)	72.4 (126)
With adjustment[3]					
Model 1: linear model with inflammation groups[4]	1564	19.6 (9.2–31.3)	32.9 (516)	11.4 (8.3–15.6)	75.6 (1182)
Model 2: linear model with CRP and AGP as continuous variables[5]	1564	17.5 (8.7–33.5)	35.4 (553)	13.1 (9.6–18.1)	86.1 (1347)
Model 3: linear models with CRP, AGP, and morbidity[6]	1564	16.0 (8.0–30.0)	38.3 (587)	12.4 (9.2–16.9)	83.6 (1305)

[1] AGP, α_1-acid glycoprotein; CRP, C-reactive protein; ID, iron deficiency; SF, serum ferritin; sTfR, soluble transferrin receptor.
[2] Cutoffs used to define ID were serum ferritin <12 μg/L and serum sTfR >8.3 mg/L.
[3] Only biomarker concentrations in individuals with inflammation (CRP >5 mg/L and AGP >1 g/L) were adjusted (n = 1070), but median and percentage ID refer to the full sample.
[4] Inflammation categories included *1*) no inflammation, *2*) CRP >5 mg/L, *3*) CRP >5 mg/L and AGP >1 g/L, and *4*) AGP >1 mg/L. Model 1 is equivalent to the correction factor approach described by Thurnham et al. (15).
[5] In the sTfR model, only CRP was significant.
[6] Morbidity variables included in the serum ferritin model were malaria, lower respiratory tract infection, and history of fever. Those in the sTfR model were malaria, fever, and acute diarrhea.

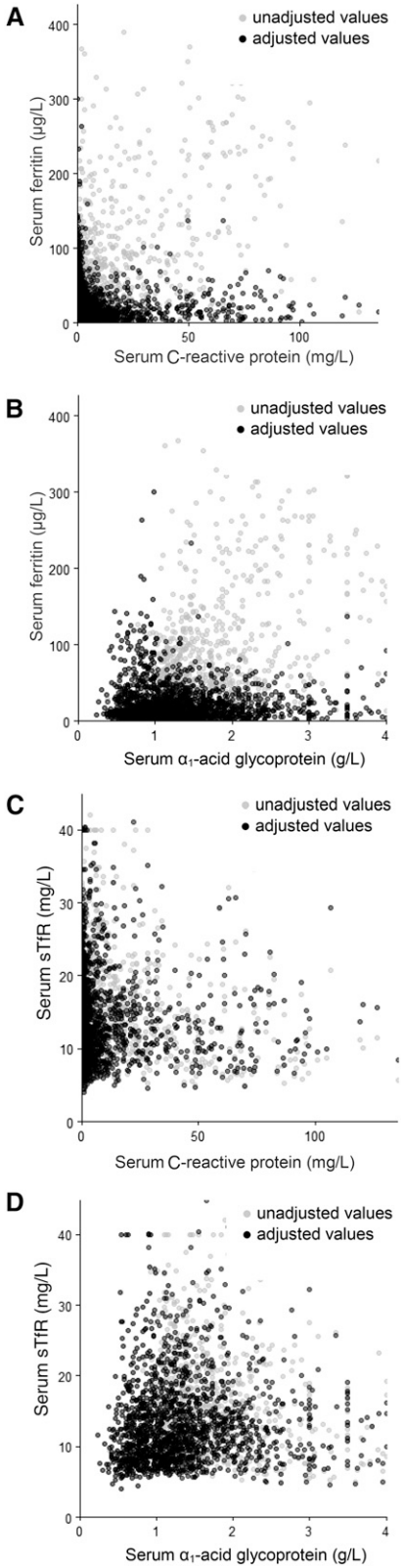

FIGURE 2 Impact of adjusting biomarker concentrations on relation with acute-phase proteins in 1564 children aged 6–23 mo in Burkina Faso. Impact of adjusting SF on relation with CRP (A); impact of adjusting sTfR on relation with CRP (B); impact of adjusting SF on relation with AGP (C); and impact of adjusting sTfR on relation with AGP (D). Gray dots indicate unadjusted SF or sTfR concentrations and black dots indicate values adjusted for inflammation. Adjusted SF and sTfR concentrations were calculated with the use of regression coefficients for CRP, AGP, and morbidity covariates from linear models predicting log-transformed SF and sTfR concentrations. AGP, α_1-acid glycoprotein; CRP, C-reactive protein; SF, serum ferritin; sTfR, soluble transferrin receptor.

estimate; however, including morbidity is not possible in the CF approach. Lastly, the estimated adjusted prevalence of ID based on linear models 2 and 3 was similar to the prevalence of ID in the subset of children without inflammation or without inflammation and infection, respectively. Overall, as expected, adjustment of SF with the use of the 3 models led to an increase in estimated prevalence of ID, which is consistent with findings of previous studies (10, 18, 20, 22).

A disadvantage of regression analysis is that it is more complex than the CF approach and requires available population data. It is unclear exactly how large a sample would be required to allow prediction models to be obtained from regression techniques, but we estimate that for sample sizes <50, the data would not be sufficiently informative.

Although we believe that regression analysis with the use of both APP and morbidity data would give a more reliable estimate of iron status and is preferable at the population level, for example, when evaluating effectiveness of interventions, it is not practical in a clinical setting for identification of ID in an individual unless the regression coefficients and devices are available to carry out the calculations. In this case, the use of a CF would be better. Interestingly, even though morbidity appears to play an important role, we found no differences in CFs calculated in apparently healthy children as part of a meta-analysis (15) compared with the ones we calculated as part of our study. In clinical settings in which a regression approach would be impractical and in which population data are not available, the use of meta-analysis CFs may therefore be appropriate to adjust SF even in children with moderate acute malnutrition.

In the case of sTfR, the models also performed better if both APPs and morbidity covariates were included. However, although in the case of SF it makes sense to pick the best-performing model for adjustment, this may not be the case for sTfR. As previously mentioned, there is still some debate as to whether sTfR should be adjusted for inflammation (10, 21–23), and there are discrepancies in the literature regarding the relation between sTfR and inflammation or infection. We found a negative relation between CRP and sTfR, as others have previously reported (6, 7). Therefore, because lower sTfR is associated with better iron status, this would suggest better iron status in individuals with elevated CRP. sTfR is a marker of erythropoiesis, as well as tissue ID (5), and lower concentrations of sTfR in children with inflammation may be a result of suppression of erythropoiesis, which occurs possibly through the actions of inflammatory cytokines (32). In contrast, we and others (9, 10, 33, 34) found higher concentrations of sTfR in individuals with malaria. It is possible that erythropoiesis is depressed during and increases shortly after the acute malaria infection stage. In line with this, it has been shown that although erythropoietin is increased in malaria (35, 36), the bone marrow response to erythropoietin may be suppressed until the parasites have been cleared (36). We measured malaria with the use of a rapid diagnostic test, which can stay positive for >1 mo following treatment (37, 38), so it is not possible to know whether a positive test reflects current or recent malaria. However, as previously mentioned, in contrast to our results, others have observed positive relations between inflammation

markers and sTfR (10–14) or shown that sTfR decreased in malaria (8). In addition to increased erythropoiesis, higher sTfR concentrations in children with infections may also be due to poorer iron status. Adjusting for morbidity may therefore lead to overadjustment. However, adjustment for CRP may be justified, because elevated concentrations of CRP in our study were associated with lower concentrations of sTfR, and inflammation may therefore lead to underestimation of ID, but the impact in our study was small. Overall, taking into consideration the inconsistencies in the literature regarding the association between sTfR and inflammation, the possible risk of overadjustment (if adjusting for morbidity as well as CRP), and the fact that the impact of adjustment was small on the whole, we believe there is no benefit from adjusting sTfR, which is in agreement with findings from other studies (21, 22).

We found a large difference in estimated prevalence of ID based on sTfR and SF, even after adjustment, which is consistent with the findings of other studies (10, 14, 22, 39, 40). Because SF and sTfR measure different aspects of iron status, differences in prevalence may not be surprising. However, the large difference in prevalence may also have other causes. First, the difference may be related to the cutoffs used. There are no internationally agreed-upon cutoffs for sTfR (4), and the appropriateness of the 12 μg/L cutoff for SF has also been questioned (41). However, although both lower (41) and higher (42) cutoffs for SF in infants under 12 mo of age have been suggested, the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition committee on nutrition concluded in a position paper that the 12 μg/L cutoff leads to over- rather than underestimation of ID (43), which would not explain the differences we found. Second, it has also been suggested that SF and sTfR may not be useful for diagnosis of ID until 9 mo of age (41), but excluding children <9 mo did not really affect prevalence of ID based on sTfR, as well as adjusted or unadjusted SF (data not shown). Furthermore, although we adjusted SF for inflammation, we did not account for the fact that children with inflammation and/or infection may also be more iron deficient than children without inflammation, and the estimated prevalence of ID after adjustment may be underestimated. Lastly, SF may also be affected by other factors, such as liver disease (44), and there may be other unknown causes of elevated sTfR in this population, such as thalassemia (45) and sickle cell anemia (5). A limitation of our study is that we did not collect data on hemoglobinopathies. A further limitation is that we were not able to compare adjustments to a gold standard for ID, namely, bone marrow iron, and it is therefore difficult to say which biomarker with which adjustment for inflammation best reflects iron status in this population.

In conclusion, regression analysis is an alternative and may be preferable to the CF approach when adjusting SF for inflammation, because it allows for accounting for severity of inflammation and morbidity, and we recommend investigating whether this approach would prove to be useful in other populations as well. However, in clinical settings, in which the regression approach would be impractical, the use of meta-analysis CFs may be appropriate. We furthermore believe that there is no benefit from adjusting sTfR. Moreover, considering the large difference in estimated prevalence of ID based on SF and sTfR, more research is needed as to which biomarker, which cutoffs for the markers, and which adjustment can best define iron status of children from low-income areas with high infectious disease burden.

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BC, CR, HF, and PK conceptualized the study; BC and CF conducted the research; BC and CR analyzed the data; and BC wrote the first draft of the manuscript and had primary responsibility for the final content. All authors revised the manuscript, and read and approved the final manuscript.

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